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Neurochemistry International 37 (2000) 403–412

NEUROCHEMISTRY International

www.elsevier.com/locate/neuint

Brain atrophy and neuronal loss in alcoholism: a role for DNA damage?

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Abstract

Chronic alcohol abuse has deleterious effects on several organs in the body including the brain. Neuroradiological studies have demonstrated that the brains of chronic alcoholics undergo loss of both gray and white matter volumes. Neuropathological studies using unbiased stereological methods have provided evidence for loss of neurons in specific parts of the brain in chronic alcoholics. The purpose of this paper is to propose a mechanism for this alcohol related neuronal loss. The hypothesis is based on the neurodegeneration observed in patients with the genetic disorder xeroderma pigmentosum (XP), who lack the capacity to carry out a specific type of DNA repair called nucleotide excision repair (NER). Some XP patients develop a progressive atrophic neurodegeneration, termed XP neurological disease, indicating that endogenous DNA damage that is normally repaired by NER has the capacity to cause neuronal death. Accumulating evidence indicates that the neurodegenerative DNA damage that is responsible for neuronal loss in XP patients results from reactive oxygen species (ROS) and lipid peroxidation products, and has the capacity to inhibit gene expression by RNA polymerase II. Therefore, the following model is proposed: chronic alcohol abuse results in increased levels of ROS and lipid peroxidation products in neurons, which results in an overwhelming burden on the NER pathway, and increased steady state levels of DNA lesions that inhibit gene expression. This results in neuronal death either by reduction in the levels of essential gene products or by apoptosis. The implications of this model for future studies are discussed.

Keywords: Oxidative damage; DNA repair; Lipid peroxidation; Acetaldehyde; Neurodegeneration

1. Introduction

Excess alcohol consumption has deleterious effects on many tissues in the body, including the brain. In a previous paper (Brooks, 1997), I presented the hypothesis that some of the toxic effects of ethanol on the body are due in part to effects on genomic DNA, and discussed the role of DNA repair in protecting against alcohol related DNA damage. The primary focus of the previous paper was on alcoholic liver disease. The present work will concentrate on the possible role of DNA damage and repair in alcohol related neuronal loss (ARNL). In doing so, I will propose a hypothesis of ARNL based on accumulation of a specific type of DNA damage. ARNL is proposed to occur via a similar mechanism as the neurodegeneration seen in patients with the hereditary disease xeroderma pigmentosum (XP), who lack the capacity to carry out a specific type of DNA repair called nucleotide excision repair (NER).

2. Evidence for alcohol related neuronal loss (ARNL) in chronic alcoholics

Chronic alcohol abuse is associated with several neurological disease states including Korsakoff's syndrome, Marchiafava–Bignami disease, pellagrous ence-

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0197-0186/00/$ - see front matter
PII: S0197-0186(00)00051-6

phalopathy, and acquired hepatocerebral degeneration (Charness, 1993; Victor, 1994). However, these diseases are not due to alcohol per se, but to nutritional deficits which occur in chronic alcoholics (Victor, 1994). In addition to the above diseases, there is evidence that the brains of alcoholics undergo toxic changes including a reduction in brain volume. In addition, there is also evidence for loss of neurons in specific regions of the brain in chronic alcoholics. I will use the term ARNL for this pathological process. ARNL undoubtedly has clinical sequeale, and may be related to the clinical entity known as alcoholic dementia or Dementia Associated with Alcoholism.

Several lines of evidence indicate that chronic alcohol abuse results in a decrease in the volume of the human brain (Harper and Kril, 1991). Some of this shrinkage is due to loss of white matter secondary to neurodegeneration (Harper and Kril, 1991; Harper, 1998). Evidence from a series of neuroimaging studies has demonstrated a progressive decrease in the volume of gray matter in the frontal lobes of chronic alcoholics, which is superimposed on a gradual decrease in the volume of frontal cortical gray matter seen during normal aging (Pfefferbaum et al., 1992, 1995, 1997). Neuroimaging studies have also shown that these volume reductions in gray matter are at least partially reversible with abstinence (Pfefferbaum et al., 1998).

Loss of gray matter in the human brain may be due to loss of neurons, shrinkage of neuronal cell bodies, or reduction in the number and extent of dendrites. Neuroimaging studies cannot distinguish between these mechanisms. The volume loss may be due to both neuron death and shrinkage, and the shrinkage may be reversible. Animal studies suggest the possibility of a combination of both atrophic cell death of a subset of neurons accompanied by sprouting of new connections in surviving neurons. Arendt et al. (1995a) have carried out a series of studies on the effect of long-term exposure to ethanol on basal forebrain neurons in rats. These authors have detected a complex response in which some neurons in the basal forebrain of ethanol drinking rats are lost, but the surviving neurons undergo a remodeling process characterized by an increase in dendritic complexity. These same authors have presented evidence for a similar process occurring in Korsakoff's disease (Arendt et al., 1995b).

Assessing the possibility that loss of gray matter volume in the brains of chronic alcoholics reflects death of neurons is a very difficult task. Meaningful data requires stringent selection criteria for both subjects and controls to exclude cases with other disease states (e.g., thiamine deficiency, liver disease). In addition, neuropathological examination of tissue sections should be carried out using appropriate quantitative techniques (i.e., unbiased stereology; Kril et al., 1997). Careful neuropathological analyses have provided evidence for both loss of neurons as well as shrinkage of individual neurons in chronic alcoholics (Harper et al., 1987; Kril and Harper, 1989; Kril et al., 1997; Kril and Halliday, 1999). Interestingly, neuronal loss is seen in certain regions of the cortex, e.g. frontal association cortex, while other regions such as the motor cortex are unaffected. A combined analysis of several cortical regions may be the reason why other authors have not detected neuronal loss in the brains of alcoholics (Badsberg-Jensen and Pakkenberg, 1993). Brain regions where neuronal loss has been demonstrated histologically roughly correspond to the regions where neuroimaging studies demonstrate the largest decreases in gray matter volume (i.e. frontal cortical regions; Kril and Halliday, 1999).

In summary, a combination of neuroimaging and histopathological data indicates that heavy alcohol consumption over a long period of time as occurs in chronic alcoholics can result in the death of neurons in specific regions of the human brain, particularly the frontal cortical regions. Careful selection criterion of subjects, along with unbiased stereological techniques, has provided strong evidence that this neuronal loss in 'uncomplicated' alcoholics is due to direct neurotoxic effects of alcohol itself, and not to other factors such as malnutrition or liver disease. It is this phenomenon, which I refer to as ARNL, that the hypothesis is intended to address.

3. Neurodegeneration in the DNA repair disease xeroderma pigmentosum

The essence of the present hypothesis is that ARNL results in part from elevated levels of a specific class of DNA damage that is normally repaired by the DNA NER pathway. To understand this hypothesis, it is useful to consider the genetic disease XP and the neurodegeneration that develops in some patients with this disorder, which is referred to as XP neurological disease.

Genomic DNA is constantly being damaged as a result of endogenous processes and the inherent chemical lability of the molecule (Lindahl, 1993). Organisms from bacteria to humans have evolved a set of DNA repair mechanisms to maintain the integrity of their genomic DNA. The five basic types of DNA repair are: (1) direct repair, carried out by the enzyme methyl guanine methyltransferase, that is responsible for removing methylation damage to guanine bases; (2) base excision repair (BER) responsible for the repair of most types of oxidative DNA damage, as well as damage from lipid peroxidation products, and methylation and deamination of DNA; (3) mismatch repair, responsible for the removal of misincorporated bases during DNA replication; (4) NER, responsible for the

repair of DNA damage from ultraviolet light, as well as other endogenous and chemical DNA adducts; and (5) recombinational repair, responsible for the repair of double strand breaks and interstrand cross-links induced by certain chemicals. The various types of DNA repair are discussed in Friedberg et al. (1995).

3.1. Xeroderma pigmentosum (XP)

The autosomal recessive disorder XP is due to a defect in the NER pathway. As described earlier, NER is involved in the repair of DNA lesions such as thymine dimers (TTDs) and 6–4 photoproducts (6–4 pps) that arise in DNA as a result of solar ultraviolet (UV) light (Friedberg et al., 1995). As a result of this inability to repair UV light induced DNA damage, XP patients are extremely sensitive to sunlight. Indeed, erythrema and sunburn from even brief sun exposure is often the first indication of the disease (Robbins et al., 1991). Moreover, because TTDs and 6–4 pps are premutageneic DNA lesions, the inability to repair these lesions results in XP patients having a greatly increased risk of developing skin cancer at an early age. The median age of onset of skin cancer in XP patients is 8 years, compared with a median age of 58 years for skin cancer in non-XP patients in the US (Cleaver and Kraemer, 1995).

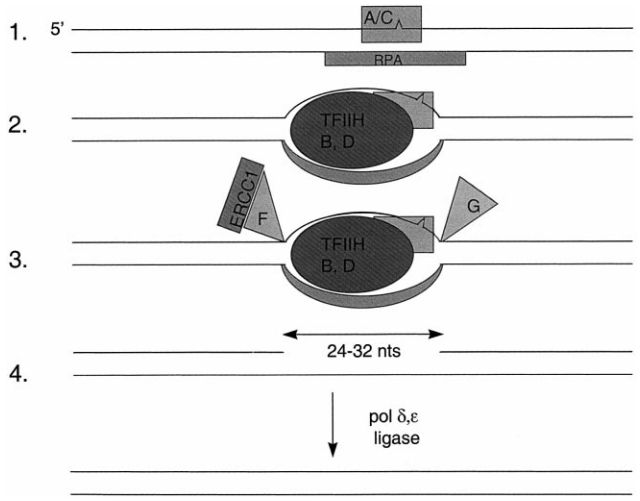

Fig. 1. A schematic diagram of the steps in NER. For simplicity, the process of NER is divided into four steps. 1. Lesion recognition: the DNA lesion (caret) is first recognized by the XP-A and/or XP-C proteins, in association with another protein, called RPA. There is at present some controversy about whether the A or the C protein is the first to recognize DNA damage (Sugasawa et al., 1998; Wakasugi and Sancar, 1999). It is clear that if the damage is on the transcribed strand of an active gene, the XP-C protein is not necessary (reviewed in Sancar, 1996; Tornaletti and Hanawalt, 1999; Wood, 1999). 2. Unwinding: this is accomplished by the helicase activities of the XPB and XPD proteins that are part of the multiprotein transcription factor IIH (TFIIH) complex. TFIIH is also necessary for transcription by RNA polymerase II. 3. Incisions: dual incisions are made asymmetrically on either side of the lesion. The XP-G protein makes the 3′ incision 2–6 nucleotides 3′ of the lesion, then the XP-F protein, in association with its partner, ERCC1, makes the 5′ incision 22–26 nucleotides 5′ to the lesion. 4. DNA synthesis and ligation: following the dual incisions, the resulting 24–32 NT gap is filled in by DNA polymerases delta and epsilon, and the remaining nick is sealed by a DNA ligase, regenerating undamaged DNA.

The structures marked A, B, C, D, F and G refer to the proteins that are mutated in XP patients in the corresponding complementation groups. The XP-V protein has recently been shown to be a novel type of DNA polymerase that has the ability to bypass thymine dimers without introducing mutations (Masutani et al., 1999). The role of the E protein is less clear, but appears to play a role in repairing some types of DNA lesions within the context of chromatin (Rapic Otrin et al., 1998). For a detailed treatment of these topics, see Sancar (1996), Wood (1999) and de Laat et al. (1999). The figure is meant to be a schematic diagram for illustrative purposes, and is not intended to literally represent specific interactions between proteins.

3.2. XP neurological disease

XP results from mutations in the genes encoding one of eight proteins, referred to as XP proteins A–G and V. The role of these different proteins in NER is schematized in Fig. 1. Of relevance to the present discussion is the fact that some XP patients, particularly those in with mutations in the genes encoding the XP-A and XP-D proteins, develop a progressive atrophic neurodegeneration, which has been termed XP neurological disease (Robbins et al., 1991). The signs and symptoms of XP neurological disease include sensorineural deafness, areflexia, microcephaly, ventricular dilation, abnormal EEG, and cerebellar and extrapyramidal disturbances (Robbins, 1988; Robbins et al., 1991). Based on neuropathological, neuroradiological, and clinical findings, the neurological abnormalities seen in XP patients are the result of a primary neurodegeneration (Robbins et al., 1991).

Upon gross inspection, the brains of patients with XP neurological disease appear atrophied, with greatly enlarged ventricles, gyri, and sulci, corresponding to a dramatic reduction in overall brain weight (Mimaki et al., 1986; Handa et al., 1985; Robbins et al., 1991). Neuropathological examination indicates a clear loss of neurons at all levels throughout the rostro-caudal extent of the brain and spinal cord (Yano, 1950; Roytta and Antitnen, 1986; Itoh et al., 1999). However, some regions and cell types are affected more than others (Yano, 1950; Roytta and Antitnen, 1986). In the cerebellum, Purkinje cells are affected while granule cells are not (Itoh et al., 1999). Motor neurons of the spinal cord and many brainstem cranial nerve nuclei are strongly affected. Degeneration is less remarkable in sensory nuclei. Dramatic losses of monaminergic cells in the locus coeruleus and substantia nigra have been reported (Yano, 1950; Roytta and Antitnen, 1986).

In the forebrain, the striatum is characterized by a

loss of the large neurons. Neurons in the basal forebrain, i.e., the substantia innominata, are also greatly reduced. Diffuse loss of neurons was also observed throughout the cerebral cortex. Some authors noted that neuronal loss was most marked in the frontal and parietal lobes, relative to other cortical regions (De Sanctis et al., 1932; Roytta and Antitnen, 1986), though Itoh et al. (1999) noted similar depletion of neurons across several cortical areas.

The age of onset and the severity of the neurodegeneration vary depending on how the mutations that each patient inherits affect the function of the mutated protein, and therefore, the repair process. In the most severe cases, such as group A patients in Japan who are homozygous for inactivating mutations in the XP-A gene, the neurodegeneration occurs at a very early age such that the patients never develop the ability to walk unaided (Maeda et al., 1995, 1997). The XP-A protein acts at an early stage of NER, acting as a sensor of damaged DNA (Fig. 1). At the other end of the spectrum, individuals with mutations in the XP-C protein develop a very mild asymptotic neurodegeneration, which can only be detected by careful audiological testing (Robbins et al., 1993) reflecting the fact the XP-C protein is not necessary for repair of DNA damage on the transcribed strand of active genes (Fig. 1).

3.3. Endogenous DNA damage and neurodegeneration in XP

Because solar UV light cannot pass through the skull and reach the brain, Robbins and colleagues (Andrews et al., 1978) proposed that the death of neurons in XP is due to the accumulation of some type of endogenous DNA damage which is normally repaired by the NER pathway. In the absence of NER, this endogenous DNA damage was hypothesized to accumulate, ultimately resulting in neuronal death due to inhibition of gene expression (Robbins et al., 1983). Following upon this hypothesis, a seminal paper by Satoh et al. (1993) demonstrated that exposure of DNA to oxygen radicals results in the generation of a class of DNA damage that is specifically repaired by the NER pathway. These authors proposed two possible classes of oxidative DNA lesions that could be specifically repaired by NER. The first type is cross-links between adjacent purines (purine dimers), which are formed by exposure of DNA to oxygen radicals (Randerath et al., 1996). The second class is the cyclopurine lesions, in which an additional bond is formed between the 5′ carbon of the deoxyribose and the eight carbon of the purine ring.

Dr. Jay Robbins and I, along with several other collaborators, are presently involved in a series of studies on the DNA repair and biological effects of 8,5′cyclodeoxyadenosine. We have found that cyclodA is a substrate for the NER pathway, but is not repaired by the base excision repair (BER) pathway, which is normally responsible for the repair of oxidative DNA damage. In addition, we have found that a single cyclodA lesion on the transcribed strand of a gene produced a 70–90% decrease in gene expression when transfected into cells lacking the ability to carry out NER (Brooks et al., 2000, in press). Based on these data, we have proposed that cyclodA is a good candidate for an endogenous DNA lesion that would accumulate in the neurons of XP patients, and contribute to the neurodegeneration observed in such patients.

In addition to the cyclo-purine lesion, there are other candidate neurodegenerative DNA lesions in XP. Detailed DNA repair and biological studies on the purine dimer lesions have not yet been carried out. Another candidate is the malondialdehyde-deoxyguanosine adduct (M1dG), which results from the reaction of the lipid peroxidation product malondialdehyde with DNA and is a substrate for repair by NER (Marnett, 1999). Notably, this adduct is present at relatively high levels in the brain (Cai et al., 1996)

In summary, the neurodegeneration observed in some XP patients has led to the conclusion that a class of endogenous DNA damage is being generated in human brain neurons that has the capacity to result in neuronal death if not repaired by the NER pathway. Several candidates for such neurodegenerative DNA lesions are presently under investigation.

4. A proposed role for DNA damage in ARNL

Based on the earlier discussion, the following hypothesis is proposed as to how accumulated DNA damage resulting from alcohol metabolism may be involved in ARNL. Specifically, the hypothesis proposes that ROS, lipid peroxidation products, and acetaldehyde (AcH) from chronic alcohol consumption increase the levels of a specific class of neurodegenerative DNA damage that is normally repaired by the NER pathway. The neurodegeneration seen in XP patients described in Section 3 provides evidence that this type of DNA damage has the capacity to kill neurons. Increased steady state levels of neurodegenerative DNA damage in neurons over decades results in ARNL as described in Section 2.

The hypothesis is primarily based on the phenotypic similarity between the neurodegeneration in chronic alcoholics and XP patients; i.e., in both conditions, what is observed is a slow loss of neurons occurring over a time course of years or decades. Moreover, in both conditions the neuronal loss occurs in the absence of any other known neurodegenerative agents such as viral infection, inflammation, spongiform encephalopa-

thy, amyloid deposits or Lewy bodies. The model is also based on the known ability of chronic alcohol consumption to increase ROS, lipid peroxidation products, and AcH, and evidence that these agents produce types of DNA damage that are repaired by the NER pathway.

A priori, the anatomical patterns of ARNL and neuronal loss in XP would not be expected to be exactly the same. In XP, neurodegeneration is believed to occur as a result of endogenous processes, specifically, DNA damage resulting from oxygen radical formation and lipid peroxidation, that are ongoing in every cell. Neurons are at particular risk in XP because of the elevated rate of oxidative metabolism, as well as the relatively low level of antioxidant defense enzymes. In contrast, the specific pattern of ARNL in frontal cortical regions is likely due in part to the brain regional distribution of components of neurotransmitter systems that are affected by ethanol. A full discussion of these systems is beyond the scope of this paper, but the GABA and glutamate systems appear to be particularly important targets for alcohol effects in the brain (for review see Breese et al., 1993; Lewohl et al., 1996; Tsai and Coyle, 1998). The specific susceptibility of the frontal cortical regions to ARNL may be due to specific alterations of the GABA-A receptor complex in this region observed in alcoholics (Lewohl et al., 1996, 1997a, 1997b).

4.1. Sources of DNA damage in the brain of chronic alcoholics

A mechanism for helix distorting DNA damage in the brain of a chronic alcoholic would be reactive oxygen species (ROS) and lipid peroxidation products. One source of increased levels of ROS would be elevated levels of the ethanol inducible CYP2E1 (Roberts et al., 1994; Hansson et al., 1990; Warner and Gustaffson, 1994). In addition, excitotoxic mechanisms involving glutamatergic neurotransmission (Iorio et al., 1993) would also result in an increase in ROS (Tsai et al., 1995, 1998; Reynolds and Hastings, 1995). Elevated levels of lipid peroxidation are seen in cells with elevated levels of CYP2E1 (Dai et al., 1993), and in the cerebrospinal fluid of human alcoholics (Tsai et al., 1998), and may lead to increased levels of the malondialdehyde–deoxyguanosine adduct. The presence of relatively high levels of this adduct in the brain has already been noted (Cai et al., 1996).

Another possible cause of helix distorting DNA damage could be AcH. Given the reactivity of AcH, it seems unlikely that AcH derived from hepatic ethanol oxidation could be transported to the brain and into the nuclei of neurons in the brain to cause significant DNA damage. However, while the brain does not have significant levels of alcohol dehydrogenase (ADH), there is evidence that oxidation of ethanol to AcH can occur in the brain (Aragon et al., 1991; Zimatkin et al., 1998). With regard to the present hypothesis, recent studies in our laboratory have shown that AcH damage to DNA produces some type of DNA damage that inhibits gene expression, and is normally repaired by the NER pathway (C. A. Marietta and P.J. Brooks, in preparation). The possible pathological significance of AcH generated by catalase in the brain deserves further investigation.

4.2. Mechanisms of cell death

In vitro studies have shown that DNA lesions that are repaired by the NER pathway are able to inhibit transcription by RNA polymerase II (Tornaletti and Hanawalt, 1999). Therefore, to the extent that lesions occur on the transcribed strand of active genes, increased steady state levels of DNA damage would result in an inhibition of expression of the affected genes, with corresponding reductions in the encoded proteins.

The hypothesis does not predict that chronic alcohol exposure would produce a level of transcription-blocking DNA damage that would be sufficient to reduce expression of every gene in every cell. Rather, the hypothesis predicts a transient reduction in a subset of genes in affected cells, and is therefore, not incompatible with increases in gene expression observed following alcohol exposure (Lewohl et al., 1997a, 1997b). Neuronal death is predicted to occur when transcription blocking DNA damage remains in genes which encode essential proteins, resulting in a reduction in protein concentration to a level that is not compatible with cell survival. Cell death in this manner, i.e. by loss of an essential gene product, would be expected to happen quite rarely, due in part to the fact that both alleles of most genes are transcribed in cells. Exceptions to this are genes affected by genomic imprinting (Bartolomei and Tilghman, 1997), and genes on the X-chromosome that are regulated by X-inactivation (Lyon, 1999). It should be borne in mind, however, that deficits in some genes could have pleotropic effects on cellular function. To take one example, alternative splicing of messenger RNAs is stimulated by a protein called SF2/ASF, and inhibited by a protein called hnRNPA1. SF2/ASF is an essential protein; loss of this protein results in cell death (Wang et al., 1996). The relative level of these two antagonistic proteins varies widely across tissues (Hanamura et al., 1998), presumably reflecting the cell-specific requirements for alternative splicing. Relatively small changes in the molar ratio of the two proteins can have dramatic effects on splicing of specific RNAs (Mayeda and Krainer, 1992), which could include RNAs encoding essential proteins.

An alternate mechanism for cell death resulting from transcription inhibiting DNA damage is apoptosis. Recent studies have shown that stalled RNA polymerases can trigger apoptosis mediated via the P53 gene product (Ljungman and Zhang, 1996; Ljungman et al., 1999). Therefore, it may be that cells have some set point for tolerating stalled polymerases, and when the number of stalled polymerases gets beyond a certain level, P53 mediated apoptosis is triggered (see also Koumenis and Giaccia, 1997).

4.3. An analogy with sun exposure, skin cell death, and skin cancer

The concept of trying to explain a pathological condition in individuals with normal levels of DNA repair based on what happens in individuals lacking a specific DNA repair pathway may at first glance seem paradoxical. However, the proposed relationship between chronic ethanol consumption and neurodegeneration is essentially analogous to the well accepted mechanism underlying skin cell death and skin cancer due to sun exposure in individuals with normal levels of NER. As described in Section 3, the NER pathway is responsible for repair of TTDs and 6–4 pps resulting from solar UV absorption by DNA. Exposure to intense sunlight results in the phenotypic appearance of sunburn, i.e. blisters formed from swollen necrotic cells. However, microscopic analysis of sunburned skin reveals a population of apoptotic keratinocytes known as sunburn cells. Experimental studies in animals have shown that these sunburn cells are not detected in mice lacking the P53 gene (Ziegler et al., 1994), and can be reduced in number by treating the skin with specific treatments which photoreverse DNA damage (Ley and Applegate, 1985). These results indicate that sunburn cells represent cells which are undergoing apoptosis as result of DNA damage that is normally repaired by NER (Ziegler et al., 1994).

Individuals with normal NER also develop skin cancer, albeit much later in life and much less frequently than XP patients. Analysis of mutations in the P53 gene of skin cancer biopsies from normal individuals has shown that mutations are clustered at tandem pyrimidines (Brash et al., 1991), characteristic of mutations arising from UV light cross links (pyrimidine dimers and 6–4 pps) (Brash et al., 1991; Ziegler et al., 1994). Thus the mechanisms underlying both sunlight associated skin cell death and skin cancer in normal individuals is the same as it is in XP patients: unrepaired UV light induced DNA damage leading to either apoptosis or mutagenesis.

The model I have presented regarding ARNL is analogous to the relationship between UV light and cell death in keratinocytes. In ARNL, the target cells are not keratinocytes but neurons; and in ARNL, the DNA damage results not from UV light but from ROS, lipid peroxidation products and perhaps AcH. One difference is that while cancers can ultimately arise following UV light exposure to the skin, brain tumors would not be expected in chronic alcoholics since tumors do not arise in terminally differentiated neurons in the mammalian brain.

5. Predictions and implications of the hypothesis

5.1. Testable predictions of the hypothesis

A model is useful to the extent that it generates experiments to test it. With regard to the present model, one prediction is that DNA damage which is normally repaired by the NER pathway will be present at higher levels in brain DNA from chronic alcoholics than in properly matched control samples. Testing this prediction will require the collection of appropriate tissue samples, from which DNA must be obtained and stored appropriately. One complication of the hypothesis as it stands is that the types of endogenous DNA adducts that are repaired by NER are at present incompletely characterized, and there are no simple assays for these lesions. However, we and others are working on developing such assays for specific lesions. One potentially useful approach to this problem is the use of the 32P post-labeling assays for DNA adducts (Steinberg et al., 1997; Randerath et al., 1999; Phillips and Castegnaro, 1999; Jones et al., 1999). While these assays are not simple, they have the advantage of assaying a variety of different forms of DNA damage at one time than most other assays, including unknown lesions. A recent publication containing a detailed standardized protocol for this type of assay has been recently published (Phillips and Castegnaro, 1999). Collaborative studies may also be useful in this regard.

There is, in fact, one intriguing case report in the literature that is consistent with the model. Steinberg et al. (1997) described an increase in a variety of incompletely characterized DNA adducts in the brain of a chronic alcoholic examined at autopsy. The authors stated that these 'neuroadducts' detected in the brain DNA of the alcoholic patient were not detected in normal brain DNA. Clearly, more studies of this type are needed to rigorously test the hypothesis.

5.2. Implications of the model: candidate genes for ARNL

There is an increasing awareness that variation in specific gene products can affect the risk of developing certain pathological outcomes. A list of genes that could affect the likelihood of developing ARNL would

obviously include those genes that affect the likelihood of becoming an alcoholic. Such candidate genes for alcoholism are discussed in other papers in this volume. Regarding alcoholic brain disease specifically, a recent report presented evidence for an effect of ADH2 genotype in differential brain atrophy in alcoholics (Maezawa et al., 1996).

The present hypothesis also has implications for candidate genes in ARNL. Several genetic variants encoding nonconservative amino acid substitutions in NER genes are present within the US Caucasian population at prevalences of greater than 1% (Shen et al., 1998). With prevalence this high, there will be substantial numbers of people who are homozygous for some of these (Mohrenweiser and Jones, 1998). If these substitutions encode functionally different proteins that affect NER, then there are significant numbers of individuals with atypical NER in the US population. If the hypothesis is correct, then such individuals may be at higher risk of developing ARNL than individuals with normal NER function.

In Japan, there is a rather different situation. The large majority of XP-A patients in Japan have one of three mutations in the XPA gene, and 80% have a splice site mutation in intron 3 which leads to a nonfunctional protein. Homozygous individuals have a severe form of the disease (Maeda et al., 1995, 1997), and due to the early onset of neurodegeneration and reduced lifespan, such patients are unlikely to face problems with alcohol. However, the prevalence of XP-A intron 3 heterozygotes in Japan has been estimated at 1:370 individuals (Maeda et al., 1997). Based on the hypothesis presented in this paper, it may be of interest to determine the XPA genotype in Japanese patients with alcohol related brain atrophy and other alcohol-related pathologies. Each of the three XPA mutations in Japan are readily assayed by restriction digestion of PCR amplified DNA (Maeda et al., 1997).

5.3. ARNL, DNA repair, aging, and thiamine deficiency

An additional aspect of the model that deserves comment is the implication of the model for the relationship between ARNL and aging. As alluded to above, alcohol effects on brain volume are superimposed upon an age related loss of gray matter volume, and there is some evidence from these studies that the aging brain may be more susceptible to the effects of alcohol (Pfefferbaum et al., 1993). Based on a review of the literature, Harper et al. (1998) concluded that there is good evidence to support a link between alcohol and aging, due to either alcohol promoting aging effects, or synergistic effects of alcohol and aging.

Regarding NER, there are several papers in the literature indicating that NER capacity decreases with age (Grossman, 1997; Wei, 1998). Many of these studies were done in peripheral tissues, but recent studies have also found similar results using brain tissues (Schmitz et al., 1999; Ploskonosova et al., 1999). Thus, it may be the case that in the older alcoholic, DNA damage from alcohol metabolism is being generated in cells that are increasingly less efficient at removing it. The net result would be higher steady-state levels of helix distorting DNA damage, with a resulting increase in the likelihood of cell death.

A final point concerns the role of liver damage. In human populations, chronic alcoholics also often suffer from liver damage as well as nutritional deficits including thiamine deficiency, which can lead to neurodegeneration (Victor, 1994). Thiamine deficiency can perturb the same neurotransmitter systems that underlie the neurotoxic effects of alcohol, i.e. the GABA (Dodd et al., 1996) and glutamate (Zhang et al., 1995) systems and result in the generation of oxygen radicals (Langlais et al., 1997). Therefore, thiamine deficiency may compound the DNA damage proposed to result from chronic alcohol as described earlier.

6. Summary

In this paper, I have presented a model of how DNA damage resulting from alcohol metabolism may be involved in ARNL. Specifically, the model proposes that ROS, lipid peroxidation products, and AcH from chronic alcohol consumption increase the levels of a specific class of neurodegenerative DNA damage that is normally repaired by the NER pathway. The neurodegeneration seen in XP patients provides evidence that this type of DNA damage has the capacity to kill neurons. Increased steady state levels of neurodegenerative DNA damage in neurons over decades results in increased neuronal death that has been described in the literature. In proposing the model, I drew an analogy between the role of NER-repairable DNA damage from UV light in skin cell death and skin cancer, and the role of NER-repairable DNA damage from endogenous products in ARNL. Stated another way, years of chronic alcohol abuse is to neurodegeneration what prolonged sunlight exposure is to skin cell death and skin cancer; a genotoxic stressor that places an additional burden on the NER pathway, increasing the likelihood of a pathological outcome. The hypothesis should now be tested by analyzing DNA from the brains of chronic alcoholics and age matched controls for increased levels of DNA damage that is normally repaired by NER. If proven correct, this hypothesis has implications for increased susceptibility of individuals with hereditary or age-related deficits in NER capacity to ARNL, and may also have treatment and prevention implications as well.

Acknowledgements

I thank Cheryl Marietta and Dr. Mary-Anne Enoch for many helpful comments on the text, members of the Laboratory of Neurogenetics, NIAAA, for useful discussions, and Dr. Jay Robbins for detailed discussions regarding XP neurological disease.

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